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THE STREPTOCOCCUS IN ACUTE EPIDEMIC RESPIRATORY INFECTION OF HORSES

SO-CALLED EQUINE INFLUENZA, STABLE FEVER, SHIPPING FEVER, EQUINE TYPHOID FEVER, CONTAGIOUS PLEUROPNEUMONIA, ETC.

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In the literature of equine medicine the term influenza is used in a general sense and serves to designate an acute infection, the etiology and relationship of which have not been determined. This or similar diseases known under such names as shipping fever, catarrhal fever, equine contagious pleuropneumonia, stable fever and typhoid fever, are especially frequent among horses subjected to wide variations in climatic environment, and the problems relating to their control are of great importance from the economic point of view. The clinical manifestations and pathologic changes of these infections vary greatly. In most instances, however, the symptoms are those of an acute inflammation of the upper respiratory tract, lungs, and lymph glands. The course of the infection is rapid and the outcome depends to a large degree on the extent of the process; the pulmonary type is perhaps the most fatal.

The etiology of equine influenza is still unknown. The fact that so-called influenza, strangles, pleuropneumonia, etc., are so closely associated in the epidemics that a differential diagnosis is usually impossible, has led many observers to believe that these diseases are all etiologically related. Streptococci are the only micro-organisms that have been found with any degree of regularity, but though constantly associated with these diseases, their etiologic importance remains to be satisfactorily determined. The investigations of Schütz,[1] Pfeiler,[2] Ferry,[3] and others are interesting in this regard. On the other hand, a filtrable virus has been mentioned as a causative factor in influenza and con-

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[1] Arch. f. Thierheilkunde, 1888, 14, p. 172; Virchows Arch., 1887, 107, p. 356.

[2] Zeitschr. f. Infektionskr. usw. Haustiere, 1910, 8, p. 155.

[3] Vet. Jour., 1912, 19, p. 185.

tagious pleuropneumonia, and in the instance of the latter, experimental evidence of a favorable nature has been brought forward by Gaffky and Lührs.[4] So far this work has not been confirmed. Observations on the etiology and relationship of equine influenza and related epidemic infections having yielded such varying results, the possibilities for valuable research in these fields would seem promising.

During the winter of 1915-1916 a widespread epidemic of acute respiratory infections occurred among horses and mules in the United States. The epidemic began in the large concentration markets in Chicago, New York, Kansas City, and St. Louis, and rapidly spread among the shipping routes to all the smaller markets throughout the country. The manifestations of this disease were striking: the onset was sudden, usually with a chill, high fever and a mucus nasal discharge. The nasal discharge rapidly became purulent and the animal appeared profoundly exhausted. In exceptional instances gastrointestinal disturbances were noted as premonitory symptoms. As a rule the course was rapid, the attack terminating favorably unless serious complications arose. Complications, however, were frequent. Pneumonia of a lobular type, but in some instances lobar, pleuritis, empyema, abscesses, suppurative lymphadenitis, arthritis, acute nephritis, and conjunctivitis were the most common complications. It was interesting that suppurative lymphadenitis and cellulitis of the neck were more frequently observed among the younger horses suffering from the disease. When death occurred some of these more severe complications were the immediate causes.

The principal changes found after death were acute hyperemia of the mucosa of the upper respiratory tract, intestinal tract and conjunctiva; marked acute hyperplasia of the lymph-nodes throughout the whole body; acute bronchopneumonia; empyema; fibrinous pleuritis and pericarditis; moderate hyperplasia of the spleen; acute parenchymatous changes in the kidneys, liver and myocardium; the embolic suppurative nephritis and abscesses in various places. In no instance were the lungs found free from inflammatory changes which varied from a limited bronchopneumonia to involvement of entire lobes. The serous membranes were frequently involved and all types of acute arthritis were observed. Inasmuch as so little is known about the etiology of such equine epidemic diseases, a bacteriologic study was made of this epidemic as it occurred in the Union Stock Yards in Chicago, and the results of the work form the basis of this communication. This work was furthered greatly by the interest and cooperation of Mr. A. G. Leonard, president of the Union Stock Yards and Transit Company.

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[4] Ztschr. f. Veterinäkunde, 1913, 1, Reviewed in Cornell Veterinarian, 1914, 4, p. 49.

TECHNIC

Cultures were made of material from the upper respiratory passages and the blood of horses at various times during the course of the infection. The material from the upper respiratory tract was collected in sterile containers and washed thoroughly through several changes of sterile normal salt solution. Stained smears were examined, and aerobic and anaerobic cultures were made on goat blood agar, ascites dextrose agar and ascites dextrose broth. All cultures were allowed to incubate 14 days before they were discarded. The bacteria obtained were identified by the usual methods. The virulence of different strains was determined and for these tests young rabbits were used.

In filtration experiments Berkefeld filters "N," and porcelain filters of the Maassen type were used. Nasal discharge or emulsion of lung tissue was macerated in a small amount of sterile salt solution before filtering. Cultures of the filtrates were made in ascites dextrose broth and ascites fluid containing a sterile piece of rabbit kidney. The ascites fluid tissue inoculations were incubated under sterile paraffin oil.

This work, which included examination of 117 horses sick or dead with the disease, yielded strikingly uniform results. In 98 horses examined before death, hemolytic streptococci were isolated from the nasal discharge in all instances. In 22 blood cultures during the course of the infection, hemolytic streptococci were found pure in 7 instances. Furthermore, these organisms were isolated from pneumonic and pleural exudates obtained during life, from the tissues about infected joints, from the urine in acute nephritis, from abscesses, iritic and conjunctival exudate, from acute nodular swellings of the extremities, and from infected lymph-nodes, in the course of the disease. Hemolytic streptococci were found regularly in infected tissues postmortem also. Other organisms such as staphylococci, green-producing streptococci, gram-negative bacilli, Bacillus subtilis and B. tetani were occasionally found in the nasal discharges. Hemolytic streptococci were the only micro-organisms found in this work with any degree of constancy. Their cultural characteristics were quite uniform. On standard blood agar plates (1 c.c. defibrinated goat blood to 9 c.c. of plain agar) the colonies were round, somewhat moist, adherent and surrounded by a clear zone of hemolysis, 2-4 mm. in diameter. In ascites dextrose broth the growth was luxuriant and the organisms settled to the bottom of the tube in the form of a white flocculent sediment. Litmus milk was acidified but not coagulated and in most instances dextrose, lactose, saccharose and salicin were fermented; many variations, however, in carbohydrate formation were observed. Anaerobic conditions were the most favorable for growth. Morphologically the organisms were variable. They were arranged in pairs, chains of pairs and single cocci, noncapsulated, and often gram-negative. The reaction to the Gram stain seemed to depend on the age of the cultures—the older cultures staining more irregularly. All the strains examined were relatively avirulent for rabbits but highly virulent for horses. Broth cultures of these organisms frequently passed through Berkefeld filters, but not porcelain filters (Maassen).

Inoculation Experiments

The results briefly described indicate that hemolytic streptococci were important elements of this epidemic disease; but other possibilities were investigated also. Was the infectious agent present in the nasal discharge, and if so, was it filtrable? To gain information on these points inoculation experiments were made. It was found that a typical attack of the disease could be produced in a normal horse by intranasal inoculation with salt solution extracts of the fresh nasal discharge of a horse sick with the infection. In 12 instances the nasal discharges of horses in the first stages of the infection and in 4 instances pieces of infected lung tissue obtained from horses dead from the disease were macerated in sterile physiologic salt solution and filtered through Berkefeld filters. The filtrates were cultured aerobically and anaerobically as well as sprayed into the noses of normal horses. In no instance did the cultures or inoculation experiments yield any evidence which would indicate the presence of a filtrable agent in the nasal discharge or lung tissue. It seemed logical therefore to assume on the basis of these results that filtrable virus either was not present in this material or it was not demonstrable by the usual methods for the study of such viruses.

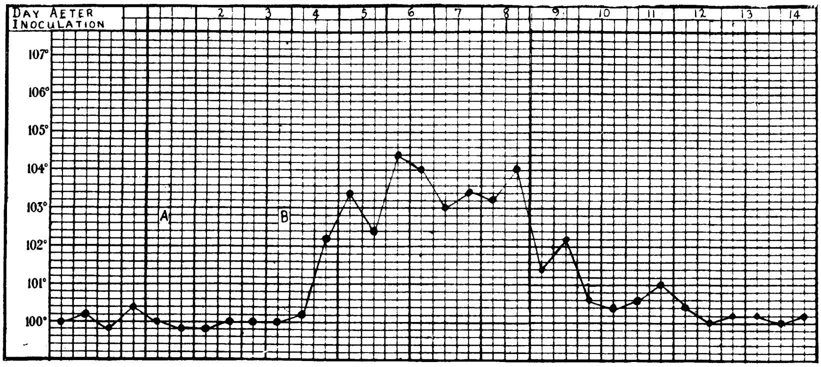

Chart 1.—The temperature curve of horse during experimental infection. *A*, horse inoculated; *B*, mucoid nasal discharge.

Three horses were inoculated intranasally and 1 horse intravenously with fresh cultures of hemolytic streptococci, isolated from the nasal discharges of horses sick with the disease. Two of the horses inoculated intranasally developed slight nasal discharges, but the general reactions following inoculation did not justify the conclusion that they were suffering from the disease. The 3rd horse so inoculated developed a typical attack of the epidemic infection 3 days after inoculation, and its record follows:

Horse 9.—A medium sized, brown gelding, 4 years old, and in good physical condition was inoculated intranasally, May 23, 1916, with a freshly isolated culture of hemolytic streptococci (Strain 19X). This culture was obtained 2 days before from the nasal discharge of a horse acutely ill with the infection. The dose used was the sediment of 180 c.c. of a 24-hour ascites-dextrose-broth culture, which was introduced with an ordinary atomizer fitted with a long rubber nozzle. The horse remained normal until May 26, when he suddenly became ill, and developed a mucus nasal discharge, fever, increased pulse and respiration. The temperature continued high for 6 days (Chart 1); on the

7th day it began to decline and gradual recovery followed and seemed complete about 18 days after the onset. There were no apparent complications other than pleuritis, the signs of which disappeared with the decline in temperature. Hemolytic streptococci were constantly present in the nasal discharge during the course of the attack, but blood cultures were sterile. The organisms isolated corresponded in every detail to the streptococci inoculated. As a control another horse was inoculated intranasally with the sterile filtrate of the nasal discharge of Horse 19X, but no signs of infections were noted as long as 1 month after the inoculation.

The horse inoculated intravenously received the bacterial sediment from 180 c.c. of 24-hour ascites-dextrose-broth culture of streptococci. Twenty-four hours later the animal developed a high temperature and all the signs and symptoms of a pleuropneumonia rapidly developed; it steadily became worse and died 8 days after the inoculation. Hemolytic streptococci were isolated from the nasal discharge, the blood, and the lung exudate obtained by puncture, before death. Postmortem examination revealed fibrinous pleuritis, acute disseminated bronchopneumonia, acute general lymphadenitis, marked acute hemorrhagic nephritis; acute parenchymatous change in the liver and myocardium. Streptococci were isolated from the lungs, kidneys, spleen, lymph-nodes and heart blood at necropsy, and found in sections of the infected tissues. A control horse was inoculated with a homologous sterile filtrate of the nasal discharge from which these streptococci were isolated, but at no time did it reveal any signs of infection.

Immunity reactions of horses, sick with this disease, with respect to streptococci have been studied in a few instances only, and the results so far are inconclusive, but this work is still in progress.

The relation of the results of this work to the clinico-anatomic characteristics of the infection is worthy of brief consideration. The acute onset, rapid course, profound exhaustion, the lobular pneumonia, the acute general hyperplasia of the lymph-nodes, and the involvement of serous membranes are all accepted characteristics of streptococcal infection. So far as this epidemic is concerned, the evidence points to hemolytic streptococci as the cause of the catarrhal inflammation of the mucous surfaces as well as of the pneumonia, and the suppurative processes. The organisms corresponded in morphology and cultural characteristics with the streptococci described by Schutz, Hel, Pfeiler and others in strangles and equine contagious pleuropneumonia, and they seemed to be highly parasitic for horses. The view that this epidemic was caused by streptococci, however, does not exclude the possibility that pneumonia and inflammations of the mucous surfaces may be caused by a variety of organisms just as in man. The streptococcus seems to bear the same pathogenic relation to the horse as to man; it is a common invader during the course of many equine infectious diseases. In the acute infections of horses in the markets this organism seems to be of primary importance, and measures for protection should be formulated accordingly. A large number of horses in transit have been inoculated with killed streptococci for purposes of protection, but the final results, which appear to be encouraging, have not yet been collected. The inoculations are being continued.

SUMMARY

During the winter of 1915-1916 an epidemic of acute infection occurred among the horses brought to the Union Stock Yards of Chicago which resembled equine influenza, or shipping fever, in its characteristics.

In a bacteriologic study of material from 117 horses sick with this disease, hemolytic streptococci were found in predominating numbers in the nasal discharges before death and infected tissues generally after death. Horses inoculated intravenously and intranasally with freshly isolated cultures of these organisms developed clinical and pathologic conditions similar to those observed in horses suffering from the epidemic disease.

The presence of pathogenic filtrable virus in material from horses sick with the infection was not demonstrated. Since hemolytic streptococci were so constantly found in predominating numbers in the nasal discharges, blood and infected tissues of horses sick with this infection, it must be concluded that they are of great importance in the etiology of the disease.